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FORM PTO 1390 (REV 10-2000) U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371		ATTORNEY'S DOCKET NUMBER P02186US0 US APPLICATION NO. (If known, see 37 CFR 1.5) 09/831546 To be assigned
INTERNATIONAL APPLICATION NO. PCT/GB99/03738	INTERNATIONAL FILING DATES 10 November 1999	PRIORITY DATE CLAIMED 10 November 1998

TITLE OF INVENTION *BACILLUS* STRAIN AND ASSAY METHODS

APPLICANT(S) FOR DO/EO/US Jeffrey Errington

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information.

1. [X] This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. [ ] This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. [ ] This is an express request to promptly begin national examination procedures (35 U.S.C. 371 (f)).
4. [X] The US has been elected by the expiration of 19 months from the priority date (PCT Article 31).
5. [X] A copy of the International Application as filed (35 U.S.C. 371 (c)(2))
   - a. [X] is attached hereto (required only if not communicated by the International Bureau).
   - b. [ ] has been communicated by the International Bureau.
   - c. [ ] is not required, as the application was filed in the United States Receiving Office (RO/US).
6. [ ] An English language translation of the International Application as filed (35 U.S.C. 371 (c)(2)).
7. [ ] Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371 (c)(3))
   - a. [ ] are attached hereto (required only if not communicated by the International Bureau).
   - b. [ ] have been communicated by the International Bureau.
   - c. [ ] have not been made; however, the time limit for making such amendments has NOT expired.
   - d. [ ] have not been made and will not be made.
8. [ ] An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371 (c)(3)).
9. [ ] An oath or declaration of the inventor(s) (35 U S C. 371 (c)(4)).
10. [ ] An English language translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371 (c)(5)).

Items 11 to 16 below concern document(s) or information included:

11. [X] An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. [ ] An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 & 3.31 is included.
13. [X] A FIRST preliminary amendment.
    [ ] A SECOND or SUBSEQUENT preliminary amendment.
14. [ ] A substitute specification.
15. [ ] A change of power of attorney and/or address letter.
16. [X] Other items or information: (a) PCT Request; (b) PCT Demand; (c) PCT Receipt of Demand; and (d) PCT Notification of Change of Address for PCT Applicant

EXPRESS MAIL LABEL NO. EK102722539US 5/10/01

09/831546 JC02 Rec'd PCT/PTO 10 MAY 2001

U.S. APPLICATION NO (if known, see 37 CFR 1.5)	INTERNATIONAL APPLICATION NO	ATTORNEY'S DOCKET NUMBER
09/831546	US99/25209	P02186US0

17. [x] The following fees are submitted: — **CALCULATIONS** PTO USE ONLY

BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) – (5)):

- [ ] Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO . . . $1000.00
- [X] International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO $860.00
- [ ] International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO $710.00
- [ ] International preliminary examination fee paid to USPTO (37 CFR 1.482) but all claims did not satisfy provisions of PCT Article 33(1)-(4) $690.00
- [ ] International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(1)-(4) $100.00

ENTER APPROPRIATE BASIC FEE AMOUNT =				$ 860.00	
Surcharge of $ 130.00 for furnishing the oath or declaration later than [ ] 20 [X] 30 months from the earliest claimed priority date (37 CFR 1.492 (e)).				$ 130.00	
CLAIMS	**NUMBER FILED**	**NUMBER EXTRA**	**RATE**		
Total claims	25 -20 =	5	x 18.00	$ 90.00	
Independent claims	1 -3 =	-0-	x 80.00	$ -0-	
MULTIPLE DEPENDENT CLAIM(s) (if applicable)			x 270.00	$ 270.00	
TOTAL OF ABOVE CALCULATIONS =				$ 1,350.00	
[ ] Applicant claims small entity status. See 37 CFR 1.27. The fees indicated above are rduced by ½.				$ 675.00	
SUBTOTAL =				$ 675.00	
Processing fee of $ ______ for furnishing the English translation later than [ ] 20 [ ] 30 months from the earliest claimed priority date (37 CFR 1.492 (f)). +				$ -0-	
TOTAL NATIONAL FEE =				$ 675.00	
Fee for recording the enclosed assignment (37 CFR 1.21 (h)). Assignment must be accompanied by appropriate cover sheet (37 CFR 3.28, 3.31) (40.00 per property). +				$ -0-	
TOTAL FEES ENCLOSED =				$ 675.00	
				Amount to be Refunded:	$
				Charged:	$

a. [ ] A check in the amount of $ ______ to cover the above fees is enclosed.

b. [X] Please charge my Deposit Account No. 06-2375 in the amount of $ 675.00 to cover the above fees. A duplicate copy of this sheet is enclosed.

c. [X] The Commissioner is hereby authorized to charge any additional fees which may be required or credit any overpayment to my Deposit Account No. 06-2375 . A duplicate copy of this sheet is enclosed.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137 (a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

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32,714
REGISTRATION NUMBER

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Serial No.:	**To be assigned**	§	**Docket No.:**	**P02186US0**
Filed:	**May 10, 2001**	§		
Applicant:	**Errington, J.**	§	**Group Unit:**	**Not yet assigned**
Title:	***Bacillus* Strain and Assay Method**	§	**Examiner:**	**Not yet assigned**

Box Patent Application
Assistant Commissioner for Patents
Washington D. C. 20231

PRELIMINARY AMENDMENT

Dear Sir:

Applicants respectfully request that a Preliminary Amendment for the above-identified Application be entered as contained herein.

In the claims:

In this patent application, please consider under prosecution claims 1-19 wherein amended claims 10-12 and 15-19 are entered as follows:

10. (Amended) A method of assessing an agent for antibiotic activity comprising the steps of:
   a) incubating the micro-organism of claim 1 in the presence of the agent; and
   b) observing expression of the reporter gene or genes.

11. (Amended) The method of claim 10, wherein the micro-organism is a *Bacillus* strain as defined in claim 4 and is induced to sporulate in the presence of the agent.

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12. (Amended) A method of determining whether an agent inhibits Spo///E function in *Bacillus* species, which method comprises inducing the *Bacillus* strain of claim 5 to sporulate in the presence of the agent, and observing expression of the first and the second reporter gene.

15. (Amended) The method of claim 11 wherein the *Bacillus* strain is induced to sporulate and is contacted, just prior to asymmetric cell division, with the agent.

16. (Amended) A panel of the micro-organisms of claim 1 wherein in different members of the panel genes have been partly or wholly replaced by homologous genes from different micro-organisms.

17. (Amended) The method of claim 10, wherein a panel of micro-organisms as defined in claim 16 is incubated in the presence of the agent, and expression of the reporter gene or genes is observed in different members of the panel.

18. (Amended) A method which comprises incubating a micro-organism of claim 1 in the presence of an agent, observing expression of the one or more reporter genes and thereby determining that the agent inhibits the growth of the micro-organisms, and using the agent as an antibiotic.

19. (Amended) A method of killing or inhibiting the growth of bacteria, which method comprises contacting the bacteria with an agent which inhibits the growth of a micro-organism of claim 1.

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REMARKS

Applicants have attached a marked version of the claims in this application. For the Examiner's convenience, Applicants have also attached a clean copy of all pending claims in this application after amendments.

Respectfully submitted,

Thomas D. Paul

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Date: 5/10/01

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MARKED VERSION OF CLAIMS SHOWING AMENDMENTS

10. A method of assessing an agent for antibiotic activity comprising the steps of:

    a) incubating the micro-organism [any one] of claim[s] 1 [to 4] in the presence of the agent; and

    b) observing expression of the reporter gene or genes.

11. (Amended) The method of claim 10, wherein the micro-organism is a *Bacillus* strain [of any one of], as defined in claim[s] 4 [to 9] and is induced to sporulate in the presence of the agent.

12. (Amended) A method of determining whether an agent inhibits SpoIIIE function in *Bacillus* species, which method comprises inducing the *Bacillus* strain of claim[s] 5 [or claim 6,] to sporulate in the presence of the agent, and observing expression of the first and the second reporter gene.

15. (Amended) The method of [any one of] claim[s] 11 [to 14], wherein the *Bacillus* strain is induced to sporulate and is contacted, just prior to asymmetric cell division, with the agent.

16. (Amended) A panel of the micro-organisms of claim 1 wherein in different members of the panel genes have been partly or wholly replaced by homologous genes from different micro-organisms.

17. (Amended) [A] The method of claim 10, wherein a panel of micro-organisms as defined in claim 16 is incubated [assessing an agent for antibiotic activity, which method comprises incubation of the members of the panel of claim 10] in the presence of the agent, and expression of the reporter gene or genes is observed in different members of the panel.

18. (Amended) A method which comprises incubating a micro-organism of [any one of] claim[s] 1 [to 9] in the presence of an agent, observing expression of the one or more reporter genes and thereby determining that the agent inhibits the growth of the micro-organisms, and using the agent as an antibiotic.

19. (Amended) A method of killing or inhibiting the growth of bacteria, which method comprises contacting the bacteria with an agent which inhibits the growth of a micro-organism of [any one of] claim[s] 1 [to 9].

CLEAN COPY OF ALL PENDING CLAIMS AS OF MAY 10, 2001

1. A micro-organism having a chromosome in which:

   a) at least one gene has been partly or wholly replaced by a homologous gene from another micro-organism; and

   b) an artificially introduced reporter gene is present and is expressed in a manner related to a homologous gene expression product.

2. The micro-organism of claim 1, where in the gene is involved in DNA replication, RNA synthesis, protein synthesis, cell wall synthesis, transport or cell division.

3. The micro-organism of claim 1 which is a bacterium.

4. The micro-organism of claim 3, wherein the bacterium is a *Bacillus* strain capable of growth and sporulation and in which at least one gene has been partly or wholly replaced by a homologous gene from another bacterium.

5. The *Bacillus* strain of claim 4, wherein:

   a) a spoIIIE gene has been replaced by its homologue from another bacterium, and

   b) two reporter genes are present each linked to a promoter and responsive to the action of σ^F during sporulation, a first reporter gene being located in a segment of the DNA that is trapped in a prespore compartment when SpoIIIE function is impaired, and a second reporter gene being located outside said segment.

6. The *Bacillus* strain of claim 5, wherein a spoIIIE gene has been partly or wholly replaced by a homologous gene from *Streptococcus pneumoniae*.

7. The *Bacillus* strain of claim 4, wherein:

   a) a cell division gene has been partly or wholly replaced by its homologue from another bacterium, and

   b) two artificially introduced reporter genes are present, a first reporter gene having a promoter which is dependent on active σ^F or σ^E factors, and a second reporter gene which provides a measure of the synthesis of the (inactive) σ^F or σ^E factor.

8. The *Bacillus* strain of claim 4, wherein the strain is modified by a mutation of a spoIIIE gene which blocks transfer of the prespore chromosome;and

   a) a spo*OJ* gene has been replaced by its homologue from another bacterium, and

   b) one or two reporter genes are present, a first reporter gene having a promoter which is dependent on σ^F factor and placed at a location where impaired Spo*OJ* function leads to increased trapping and hence to increased expression in the prespore, and/or a second reporter gene having a promoter which is dependent on σ^F and placed at a location where impaired Spo*OJ* function leads to reduced trapping and hence to reduced expression in the prespore.

9. The *Bacillus* strain of any one of claims 4 to 8, which is a *B. subtilis* strain.

10. A method of assessing an agent for antibiotic activity, which comprises the steps of:

a) incubating the micro-organism of claim 1 in the presence of the agent; and

b) observing expression of the reporter gene or genes.

11. The method of claim 10, wherein the micro-organism is a *Bacillus* strain as defined in claim 4 and is induced to sporulate in the presence of the agent.

12. A method of determining whether an agent inhibits Spo///E function in *Bacillus* species, which method comprises inducing the *Bacillus* strain of claim 5 to sporulate in the presence of the agent, and observing expression of the first and the second reporter gene.

13. A method of determining whether an agent inhibits cell division in *Bacillus* species, which method comprises inducing the *Bacillus* strain of claim 7 to divide asymmetrically, as during sporulation, in the presence of the agent, and observing expression of the first and second reporter genes.

14. A method of determining whether an agent inhibits Spo*OJ* function in *Bacillus* species, which method comprises inducing the *Bacillus* strain of claim 8 to divide

asymmetrically, as during sporulation, in the presence of the agent, and observing expression of the first and/or the second reporter gene.

15. The method of claim 11 wherein the *Bacillus* strain is induced to sporulate and is contacted, just prior to asymmetric cell division, with the agent.

16. A panel of the micro-organisms of claim 1 wherein in different members of the panel genes have been partly or wholly replaced by homologous genes from different micro-organisms.

17. The method of claim 10, wherein a panel of micro-organisms as defined in claim 16 is incubated in the presence of the agent, and expression of the reporter gene or genes is observed in different members of the panel.

18. A method which comprises incubating a micro-organism of claim 1 in the presence of an agent, observing expression of the one or more reporter genes and thereby determining that the agent inhibits the growth of the micro-organisms, and using the agent as an antibiotic.

Docket No.: P02186US0

19. A method of killing or inhibiting the growth of bacteria, which method comprises contacting the bacteria with an agent which inhibits the growth of a micro-organism of claim 1.

BACILLUS STRAIN AND ASSAY METHOD

Whole-cell assays are known for specific inhibitors of *B. subtilis* proteins involved in chromosome partitioning and cell division. The property, of inhibiting chromosome partitioning and cell division, is indicative of actual or potential anti-microbial properties. The inventor has devised three such assays; they are described in WO 97/00325; WO 98/26087; and WO 98/26088, which are summarised below and to which reference is directed.

New compounds inhibitory for any chromosome partitioning and cell division functions are likely to have a broad spectrum of activity against a wide range of bacteria, including important pathogens, because the functions targeted appear to be highly conserved. However, it is possible that some of the compounds discovered may turn out to be relatively specific for the *B. subtilis* proteins, in which case they would not be useful general purpose antimicrobial agents.

A similar problem arises in any whole-cell assay for an inhibitor of a specific gene of any micro-organism. The problem is that an inhibitor of a specific gene of a particular strain or micro-organism, may be specific to that strain, or alternatively may have inhibitory properties which are exerted over a rather wide range of micro-organisms. The present invention addresses that problem by replacing a target gene in a micro-organism used for a whole-cell assay with a homologous gene from a different organism, e.g. a micro-organism of more direct interest.

Thus the invention provides in one aspect a micro-organism having a chromosome in which:

a) at least one gene has been partly or wholly replaced by a homologous gene from another micro-organism, and

b) an artificially introduced reporter gene is present and is

expressed in a manner related to a homologous gene expression product.

In another aspect the invention provides a method of assessing an agent for antibiotic activity, which method comprises incubating the micro-organism as defined in the presence of the agent, and observing expression of the reporter gene or genes.

The micro-organism may be for example a yeast or more preferably a bacterium. The bacterium may be a *Bacillus* species that is capable of growth and sporulation under suitable conditions and for which genetic constructs can be made. *B. subtilis* is conveniently accessible and well characterised and is preferred.

A homologous gene is a functionally equivalent gene from another micro-organism. In the micro-organism of the present invention, at least one gene (the target gene) has been partly or wholly replaced by a homologous gene from another micro-organism. Preferably the target gene is one which is well conserved over many different species of bacteria or other micro-organisms. It is necessary that the homologous gene be functionally incorporated so as to be capable of expression *in vivo*. When the target gene is partly or wholly replaced by a homologous gene, it is necessary that the homologous gene be capable of forming an expression product that is different in some respect from the expression product of the target gene. Suitable target genes include genes involved in DNA replication, RNA synthesis, protein synthesis, cell wall synthesis, transport and cell division.

For micro-organisms which are Bacillus species e.g. *B. subtilis*, cell division genes include *divIB* (also called *ftsQ*), *divIC, divIVA, ftsA, ftsL* (also called *mraR*), *ftsZ, pbpB*, as well as *spoOJ* and *spoIIIE*, and others, both known and to be discovered. Since these cell division genes are substantially conserved across many bacterial species, it is plausible that these engineered *Bacillus* strains will grow and sporulate with reasonable efficiency. The homologous gene may be taken from other bacilli or closely related organisms such as *clostridia* and *Listeria*. More

preferably, the homologous gene may be taken from a pathogenic bacterium such as staphylococci and streptococci. *B. subtilis* molecular genetic methods make it straightforward to replace any gene with a homologous gene from another bacterium.

An artificially introduced reporter gene is one which is not naturally present in the strain in question, and which may have been introduced by genetic manipulation. A reporter gene is one which on expression gives rise to an easily detected or observed phenotype. For example, the expressed protein may be an enzyme which acts on a substrate to give a product that is easily observed e.g. because it is coloured or chemiluminescent of fluorescent. Reporter genes capable of being expressed in *Bacillus* species and other micro-organisms are well known and documented in the literature. Reporter genes are preferably chosen so that their products can be readily assayed simultaneously. *lacZ* has been used for more than 10 years with great success in *B. subtilis* and there is a range of useful substrates that generate coloured or fluorescent products upon hydrolysis by ß-galactosidase. The *uidA* gene of *E. coli* has recently been harnessed for similar purposes, and the range of substrates available for the gene product, ß-glucoronidase is similar to that for ß-galactosidase.

In one example, two different fluorogenic substrates are used to assay the activities of the two reporters simultaneously in a single reaction.

On incubation of the micro-organism, e.g. on cell division or sporulation, a reporter gene is expressed in a manner related to the activity of an expression product e.g. a cell division protein, of the homologous gene. For example, decreased activity of that protein may be associated with either increased expression or reduced expression of the reporter gene. When two reporter genes are used, preferably expression of one is increased, and expression of the other is decreased, in association with a change in the level of activity of that protein.

The preferred assay method of the invention involves inducing the *Bacillus* strain described to sporulate in the presence of a putative anti-microbial agent. Preferably the *Bacillus* strain is contacted, just prior to asymmetric cell division with the agent. To screen agents on a large scale, samples of the *Bacillus* strain may be cultured in an exhaustion medium to stimulate sporulation; either in the wells of a microtitre plate to which the agent is added; or in bulk to be dispensed into the wells of a microtitre plate of which individual wells contain one or more different agents. After suitable incubation, observation is made of expression of the one or more reporter genes. For example, when the expression products of two reporter genes are different enzymes, substrates for the two enzymes may be added to the wells of the microtitre plate, and observation made of e.g. chemiluminescent or fluorescent or coloured products of enzymatic activity.

Use of such strains have several practical consequences:

i) It enables inhibitors which act on the protein product of a pathogen but not on that of a parent micro-organism e.g. *B. subtilis* to be identified.

ii) In the case of an assay for inhibitors of cell division, it may facilitate identification of the specific target of the inhibitor. By screening promising compounds against a series of strains in which cell division genes have been systematically replaced with homologues from other organisms, the specific target of the inhibitory compound becomes evident. Thus, for example, detection of a compound which inhibits the *B. subtilis* parent strain but not a derivative carrying the *S. aureus* homologue of *ftsZ*, would be strongly suggestive of a compound targeted on the FtsZ protein.

iii) A panel of strains with a given target gene systematically replaced by genes from other organisms also provides information about the spectrum of activity of each potential inhibitor. For example, some of the compounds found to inhibit the *B. subtilis* SpoIIE protein might not act on the strain bearing its *S. aureus* homologue. Other compounds might

show non-species specific inhibition and act on a range of gene products from different organisms. Such tests provide a useful means of ensuring that new inhibitors have a broad spectrum of activities.

Thus in another aspect the invention provides a panel of the micro-organisms as defined, wherein in different members of the panel genes have been partly or wholly replaced by homologous genes from different micro-organisms. The invention also includes a method of assessing an agent for antibiotic activity, which method comprises incubating the members of the panel in the presence of the agent, and observing expression of the reporter gene or genes in different members of the panel. Also provided, is a method of killing or inhibiting the growth of bacteria, which method comprises contacting the bacteria with an agent which inhibits the growth of a micro-organism according to the invention.

According to **WO 97/00325**, a unique sporulation phenotype arising when *spoIIIE* is inactivated provides the potential for a very powerful and specific assay. In the absence of functional *spoIIIE*, the chromosome is trapped partially inside and partially outside the prespore compartment, but the prespore-specific transcription factor σ^F is activated normally. Reporter genes dependent on σ^F are expressed if they are located at certain places in the chromosome and blocked if they lie elsewhere. That invention provides a *Bacillus* strain having a chromosome with two reporter genes each linked to a promoter and responsive to the action of σ^F during sporulation, a first reporter gene being located in a segment of the DNA that is trapped in a prespore compartment when *spoIII* function is impaired, and a second reporter gene being located outside the said segment. An assay method using the *Bacillus* strain is also described.

The *B. subtilis spoIIIE* gene is required for translocation of the prespore chromosome through an asymmetrically positioned septum during sporulation in *B. subtilis*. Although at first sight this appears to be a very specialised mechanism, *spoIIIE*-like genes are highly conserved throughout bacteria. A more general function for the *B. subtilis* gene was revealed by

experiments in which wild type and *spoIIIE* mutant cells of *B. subtilis* were exposed to sub-lethal concentrations of inhibitors of DNA replication (Sharpe and Errington, 1995, Proc. Natl. Acad. Sci. USA **92**, 8630-8634). Under such conditions the probability of chromosomal DNA being caught in the division septum is increased. Wild type cells could recover from this state but in *spoIIIE* mutants the chromosome remained trapped and so these mutants were more sensitive to such inhibitors.

If removal of chromosomal DNA from the division septum is the general function of SpoIIIE protein, and its action during sporulation just an extreme manifestation of this function, the *spoIIIE*-like genes from non-sporulating bacteria might be able to functionally complement the defect of *spoIIIE* mutants of *B. subtilis* and restore their ability to sporulate.

According to the present invention, the *spoIIIE* gene is partly or wholly replaced by a homologous gene from another bacterium. The use of the homologous gene from *Streptococcus pneumoniae* is described in the example below.

According to **WO 98/26087**, the effects of *spoOJ* mutations on prespore chromosome orientation, and the ability to detect this by use of a *spoIIIE* mutant background, provides the potential for a very specific whole-cell assay for inhibitors of *spoOJ* function. The presence of any given segment of chromosomal DNA in the prespore can be detected by use of a reporter gene controlled by a transcription factor σ^F, which is activated only in the small prespore compartment (a process that is not affected by perturbations in chromosome partitioning).

WO 98/26087 thus provides a *Bacillus* strain having a chromosome with the following modifications:

a) a mutation of a *spoIIIE* gene which blocks transfer of the prespore chromosome,

b) a mutation in the *soj* gene which prevents loss of *spoOJ* function from blocking sporulation, together with

c) a first reporter gene having a promoter which is dependent on

σ^F factor and placed at a location where impaired *spoOJ* function leads to increased trapping and hence to increased expression from the prespore, and/or

d) a second reporter gene having a promoter which is dependent on σ^F factor and placed at a location where impaired *spoOJ* gene function leads to reduced trapping and hence to reduced expression in the prespore.

The present invention provides a *Bacillus* strain of this kind in which the *spoOJ* gene has been replaced by its homologue from another bacterium.

Synthesis of σ factor begins at the onset of sporulation, but its product is initially held in an inactive state by the action of an anti-σ factor *spoIIAB*. Release from inhibition requires the concerted action of at least two other proteins, *SpoIIAA* and *SpoIIE*, which serve to allow release of σ^F activity only after the sporulating cell has undergone asymmetric cell division and to restrict the σ^F activity to the smaller prespore cell type. According to **WO 98/26088**, this dependence of σ^F activation on septation is used as the basis for a sensitive assay for inhibitors of cell division. Thus that specification provides a *Bacillus* strain having two reporter genes, a first reporter gene having a promoter which is dependent on active σ^F (or σ^E), and a second reporter gene having a promoter regulated similarly to the gene encoding the σ factor, to provide a measure of the synthesis of the (inactive) σ factor. A whole-cell screening method for identifying antimicrobial agents involves use of the *Bacillus* strain.

According to the present invention, any *Bacillus* cell division gene involved in these activities is partly or wholly replaced by a homologous gene from another bacterium. The *Bacillus* gene may be for example *divIB* (also called *ftsQ*), *divIC, divIVA, ftsA, ftsL* (also called *mraR*), *ftsZ, pbpB*, as well as *spoOJ* and *spoIIIE*.

Example

The inventors constructed a strain of *B. subtilis* in which the final 310 amino acid residues of the *spoIIIE* gene had been replaced with the equivalent section of the gene from *Streptococcus pneumoniae* strain R6, either in the correct or inverted orientation. In the correct orientation, the strain should make a hybrid protein comprising the poorly conserved membrane anchor region encoded by the *B. subtilis* gene fused to the highly conserved C-terminal coding region of the *Streptococcus pneumoniae* gene.

Strains with the *Streptococcus* gene inserted in either the correct or the inverted orientation, relative to the host *spoIIIE* gene, were induced to sporulate by a standard resuspension method, in parallel with an isogenic wild type strain. After 9 hours, the number of spores formed was measured on the basis of heat resistance, by heating at 80°C for 10 min and then plating serial dilutions on nutrient agar. Colonies were counted after overnight incubation. In the strain with the *Streptococcus* DNA inserted in the inverted orientation, and so with no intact *spoIIIE* gene, sporulation was completely abolished (< 10 heat resistant colony forming units [cfu] per ml of culture). However, with the *Streptococcus* DNA in the correct orientation, spore heat resistance was found to arise with approximately equal frequency (2.0×10^8 cfu per ml) to the wild type (1.3×10^8 cfu per ml). The outgrowth of a new colony from a heat resistant spore requires that the spore had acquired a complete chromosome. Thus, the hybrid gene must have been able to catalyse chromosome transfer into the spore compartment just as well as the wild type SpoIIIE protein.

CLAIMS

1. A micro-organism having a chromosome in which:

a) at least one gene has been partly or wholly replaced by a homologous gene from another micro-organism, and

b) an artificially introduced reporter gene is present and is expressed in a manner related to a homologous gene expression product.

2. The micro-organism of claim 1, wherein the gene is involved in DNA replication, RNA synthesis, protein synthesis, cell wall synthesis, transport or cell division.

3. The micro-organism of claim 1 or claim 2, which is a bacterium.

4. The micro-organism of claim 3, wherein the bacterium is a *Bacillus* strain capable of growth and sporulation and in which at least one gene has been partly or wholly replaced by a homologous gene from another bacterium.

5. The *Bacillus* strain of claim 4, wherein:

a) a *spoIIIE* gene has been replaced by its homologue from another bacterium, and

b) two reporter genes are present each linked to a promoter and responsive to the action of σ^F during sporulation, a first reporter gene being located in a segment of the DNA that is trapped in a prespore compartment when SpoIIIE function is impaired, and a second reporter gene being located outside the said segment.

6. The *Bacillus* strain of claim 5, wherein a *spoIIIE* gene has been partly or wholly replaced by a homologous gene from *Streptococcus pneumoniae*.

7. The *Bacillus* strain of claim 4, wherein:

a) a cell division gene has been partly or wholly replaced by its homologue from another bacterium, and

b) two artificially introduced reporter genes are present, a first reporter gene having a promoter which is dependent on active σ^F or σ^E factors, and a second reporter gene which provides a measure of the synthesis of the (inactive) σ^F or σ^E factor.

8. The *Bacillus* strain of claim 4, wherein the strain is modified by a mutation of a *spoIIIE* gene which blocks transfer of the prespore chromosome, and:

a) a *spoOJ* gene has been replaced by its homologue from another bacterium, and

b) one or two reporter genes are present, a first reporter gene having a promoter which is dependent on σ^F factor and placed at a location where impaired S*poOJ* function leads to increased trapping and hence to increased expression in the prespore, and/or a second reporter gene having a promoter which is dependent on σ^F factor and placed at a location where impaired *SpoOJ* function leads to reduced trapping and hence to reduced expression in the prespore.

9. The *Bacillus* strain of any one of claims 4 to 8, which is a *B. subtilis* strain.

10. A method of assessing an agent for antibiotic activity, which method comprises incubating the micro-organism of any one of claims 1 to 4 in the presence of the agent, and observing expression of the reporter gene or genes.

11. The method of claim 10, wherein the *Bacillus* strain of any one of claims 4 to 9 is induced to sporulate in the present of the agent.

12. A method of determining whether an agent inhibits SpoIIIE function in *Bacillus* species, which method comprises inducing the *Bacillus* strain of claim 5 or claim 6, to sporulate in the presence of the agent, and observing expression of the first and the second reporter genes.

13. A method of determining whether an agent inhibits cell division in *Bacillus* species, which method comprises inducing the *Bacillus* strain of claim 7 to divide asymmetrically, as during sporulation, in the presence of the agent, and observing expression of the first and second reporter genes.

14. A method of determining whether an agent inhibits SpoOJ function in *Bacillus* species, which method comprises inducing the *Bacillus* strain of claim 8 to divide asymmetrically, as during sporulation, in the presence of the agent, and observing expression of the first and/or the second reporter gene.

15. The method of any one of claims 11 to 14, wherein the *Bacillus* strain is induced to sporulate and is contacted, just prior to asymmetric cell division, with the agent.

16. A panel of the micro-organisms of any one of claims 1 to 9, wherein in different members of the panel genes have been partly or wholly replaced by homologous genes from different micro-organisms.

17. A method of assessing an agent for antibiotic activity, which method comprises incubation of the members of the panel of claim 16 in the presence of the agent, and observing expression of the reporter gene or genes in different members of the panel.

18. A method which comprises incubating a micro-organism of any one of claims 1 to 9 in the presence of an agent, observing expression of the one or more reporter genes and thereby determining that the agent inhibits the growth of the micro-organisms, and using the agent as an antibiotic.

19. A method of killing or inhibiting the growth of bacteria, which method comprises contacting the bacteria with an agent which inhibits the growth of a micro-organism of any one of claims 1 to 9.

INVENTOR DECLARATION	Attorney Docket No. P0~~2077~~US0 2186

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled BACILLUS STRAIN AND ASSAY METHOD, the specification of which

(check one) ☐ is attached hereto.

☒ was filed on 10 May 2001 as Application Serial No. 09/831,546 (if applicable)

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, § 1.56(a).

I hereby claim foreign priority benefits under Title 35, United States Code, § 119(a)-(d) or § 365(b) of any foreign application(s) for patent or inventor's certificate, or § 365(a) of any PCT international application which designated at least one country other than the United States of America, listed below and have also identified below any foreign application for patent or inventor's certificate, or of any PCT international application having a filing date before that of the application on which priority is claimed:

Prior Foreign Application(s)

(Number)	(Country)	(Day/Month/Year Filed)	Priority Claimed Yes	Priority Claimed No
9824682.0	United Kingdom	10 November 1998	☒	☐
			☒	☐
			☐	☐

I hereby claim the benefit under Title 35, United States Code § 119(e) of any United States provisional application(s) listed below:

(Application Serial No.)	(Filing Date)

I hereby claim the benefit under Title 35, United States Code §120 of any United States application(s), or § 365(b) of any PCT international application designating the United States of America, listed below and insofar as the subject matter of each of the claims of this application is not disclosed in the prior U.S. or PCT international application in the manner provided by the first paragraph of Title 35, U.S.C. §112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations §1.56(a) which occurred between the filing date of the prior application and the national or PCT international filing date of this application.

(Application Serial No.)	(Filing Date)	(Status) (patented, pending, abandoned)
PCT/GB 99/03738	10 November 1999	

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

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POWER OF ATTORNEY

ISIS INNOVATION LIMITED, assignee of all right, title and interest in the enclosed patent application Serial No. 09/831,546, filed 10 May 2001, hereby appoints the following agent(s) to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith:

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22

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